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Figure 1

Sequence alignment of mouse Serca 1, 2 and 3 protein.

5	Serca1a	1	MEAAHSKSTEECLSYFGVSETTGLTPDQVKRHLEKYGNELPAEEGKSLWELVVEQFEDL
	Serca2a	1	..N..T.TV..V.GH...N.S...SLE...KLK.RW.S.....T.L...I.....
	Serca2b	1	..N..T.TV..V.GH...N.S...SLE...KLK.RW.S.....T.L...I.....
	Serca3a	1	..E..LL.AADV.RR.S.TAEG..SLE..TDAR.R.....T.....
	Serca3b	1	..E..LL.AADV.RR.S.TAEG..SLE..TDAR.R.....T.....
10	Serca3c	1	..E..LL.AADV.RR.S.TAEG..SLE..TDAR.R.....T.....
	Serca1a	61	LVRILLLAACISFVLAWFEEGEETVTAFFVEPFVILLILIANAIVGVWQERNAENAIEALK
	Serca2a	61I.....V.....
	Serca2b	61I.....V.....
15	Serca3a	61LV.....T.....L..M..V.....S.....
	Serca3b	61LV.....T.....L..M..V.....S.....
	Serca3c	61LV.....T.....L..M..V.....S.....
	Serca1a	121	EYEPENMGKVYRADRKSVQRIKARDIVPGDIVEVAVGDKVPADIRILSIKSTTLRVDQSIL
20	Serca2a	121Q.....K.....I.....LT.....
	Serca2b	121Q.....K.....I.....LT.....
	Serca3a	121I.S...G...R.....L.LIE.....
	Serca3b	121I.S...G...R.....L.LIE.....
	Serca3c	121I.S...G...R.....L.LIE.....
25	Serca1a	181	TGESVSVIKHTDPVPDPRAVNQDKKNMLFSGTNIAAGKAVGIVATTGVSTEIGKIRDQMA
	Serca2a	181M.V.VA...N.....E.V
	Serca2b	181M.V.VA...N.....E.V
	Serca3a	181T...AI.....S...L.VAVA..LQ..L...S...
30	Serca3b	181T...AI.....S...L.VAVA..LQ..L...S...
	Serca3c	181T...AI.....S...L.VAVA..LQ..L...S...
	Serca1a	241	ATEQDKTPLQOKLDEFGEQLSKVISLICVAVWLNIGHFNDPVHGGSWFRGAIYYFKIAV
35	Serca2a	241ER.....I...I.....I.....
	Serca2b	241ER.....I...I.....I.....
	Serca3a	241	.V.PER...R.....R..HA..V.....V.....A..A....L..V.....
	Serca3b	241	.V.PER...R.....R..HA..V.....V.....A..A....L..V.....
	Serca3c	241	.V.PER...R.....R..HA..V.....V.....A..A....L..V.....
40	Serca1a	301	ALAVAAIPEGLPAVITTCLALGTRMAKKNNAIVRSLPSVETLGCTSVICSDKTGTLTTNQ
	Serca2a	301
	Serca2b	301
	Serca3a	301R.....
	Serca3b	301R.....
45	Serca3c	301R.....
	Serca1a	361	MSVCKMFIIDKVDGDCSLNEFSITGSTYAPEGEVLKNDKPVVRAGQYDGLVELATICALC
	Serca2a	361R...L...E..T.....I...Q.D...KCH.....
	Serca2b	361R...L...E..T.....I...Q.D...KCH.....
50	Serca3a	361R...VVAEAEAGT.R.H..T.S.T..T....RQGEQ...C..F.....
	Serca3b	361R...VVAEAEAGT.R.H..T.S.T..T....RQGEQ...C..F.....
	Serca3c	361R...VVAEAEAGT.R.H..T.S.T..T....RQGEQ...C..F.....
	Serca1a	421	NDSSSLDFNETKGVYEKVGATETALTTLVEKMNVFNTVRSLSKVERANACNSVIRQLMK
55	Serca2a	421	...A..Y..A.....C.....D..LKG...I.....K....
	Serca2b	421	...A..Y..A.....C.....D..LKG...I.....K....
	Serca3a	421	...A..Y..A.....C.....D..DLKG..R....G.....K...R
	Serca3b	421	...A..Y..A.....C.....D..DLKG..R....G.....K...R
	Serca3c	421	...A..Y..A.....C.....D..DLKG..R....G.....K...R

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	Serca1a	481	KEFTLEFSRDRKSMSVYCSPAKSSRAAVGNKMFVKGAPEGVIDRCNYVRVGTTTRVPLTGP
	Serca2a	481T.N.P..TSMS-.....THI...S.K..M.PG
	Serca2b	481T.N.P..TSMS-.....THI...S.K..M.PG
	Serca3a	481T.TRADPKVQ.S.....S..E..SS....SRTA..STT
5	Serca3b	481T.TRADPKVQ.S.....S..E..SS....SRTA..STT
	Serca3c	481T.TRADPKVQ.S.....S..E..SS....SRTA..STT
	Serca1a	541	VKEKIMSVIKWGTGRDRTLRLCLALATRDTPPKREEMVLDDSAKFMEYEMDLTFVGVVGML
	Serca2a	540	..Q.....R...S.S.....H.N.L.....H.E...N.IK..TN....C....
10	Serca2b	540	..Q.....R...S.S.....H.N.L.....H.E...N.IK..TN....C....
	Serca3a	541	SR.H.LAK.RD..S.S.....RK.D.H...CSR.VQ..T.....C....
	Serca3b	541	SR.H.LAK.RD..S.S.....RK.D.H...CSR.VQ..T.....C....
	Serca3c	541	SR.H.LAK.RD..S.S.....RK.D.H...CSR.VQ..T.....C....
15	Serca1a	601	DPPRKEVTGSIQLCRDAGIRVIMITGDNKGTAIAICRRIGIFSENEEVTDRAYTGREFDD
	Serca2a	600I..AS.VK...Q.....V.....GQD.D..SK.F.....E
	Serca2b	600I..AS.VK...Q.....V.....GQD.D..SK.F.....E
	Serca3a	601P..AAC.TR.SR....V.....V.....L...GDT.D.LGK.....
	Serca3b	601P..AAC.TR.SR....V.....V.....L...GDT.D.LGK.....
20	Serca3c	601P..AAC.TR.SR....V.....V.....L...GDT.D.LGK.....
	Serca1a	661	LPLAEQREACRRACCFARVEPSHKSKIVEYLQSYDEITAMTGDGVNDAPALKKAEIGIAM
	Serca2a	660	.SPSA..D..LN.R.....F..F.....S.....
	Serca2b	660	.SPSA..D..LN.R.....F..F.....S.....
25	Serca3a	661	.SPEQ..Q...T.R.....A...R...N...FN.....
	Serca3b	661	.SPEQ..Q...T.R.....A...R...N...FN.....
	Serca3c	661	.SPEQ..Q...T.R.....A...R...N...FN.....
	Serca1a	721	GSGTAVAKTASEMVLADDNFSTIVAAVEEGRAIYNNMKQFIRYLISSNVGEVVCIFLTAA
30	Serca2a	720
	Serca2b	720
	Serca3a	721S.A...S...AS.....I
	Serca3b	721S.A...S...AS.....I
	Serca3c	721S.A...S...AS.....I
35	Serca1a	781	LGLPEALIPVQLLWVNLVTDGLPATALGFNPPDLDIMDRPPRSPEPLISGWLFFRYMAI
	Serca2a	780	..F.....NK...N.....L..
	Serca2b	780	..F.....NK...N.....L..
	Serca3a	781EK...N.R.A.....L..
40	Serca3b	781EK...N.R.A.....L..
	Serca3c	781EK...N.R.A.....L..
	Serca1a	841	GGYVGAATVGAAAWFLYAEDGPHVSYHQLTHFMQCTEHNPEFDGLDCEVFPEPEPMTMA
	Serca2a	840	.C.....IA.DG..R..FY..S..L..K.D..D...V..AI..S.Y.....
45	Serca2b	840	.C.....IA.DG..R..FY..S..L..K.D..D...V..AI..S.Y.....
	Serca3a	841	.V...L...A..T....DAE..Q.TFY..RN.LK.S.D..L.A.I..K...SRF.T...
	Serca3b	841	.V...L...A..T....DAE..Q.TFY..RN.LK.S.D..L.A.I..K...SRF.T...
	Serca3c	841	.V...L...A..T....DAE..Q.TFY..RN.LK.S.D..L.A.I..K...SRF.T...
50	Serca1a	901	LSVLVTIEMCNALNSLSENQSLLRMPWVNIWLLGSICLSMSLHFLILYVDPLPMIFKLR
	Serca2a	900E....V.....E...L..QIT
	Serca2b	900E....V.....E...L..QIT
	Serca3a	901V.....L.P....AVVM..A.....L.P...L..QVT
	Serca3b	901V.....L.P....AVVM..A.....L.P...L..QVT
55	Serca3c	901V.....L.P....AVVM..A.....L.P...L..QVT

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Serca1a 961 ALDFTQWLMVLKISLPVIGLDELLKFIARNYLEG
Serca2a 960 P.NL.....LM..T...V.....QPATLE
Serca2b 960 P.NL.....LM..T...V.....QPGKECVQPATKSSCSLSACTDGISWP
Serca3a 961 P.SGR..GV..QM.....L...A..YLS..HMDEKKDLK
5 Serca3b 961 P.SGR..GV..QM.....L...A..YLS..HMD.VLGTFMQARSROLPTTSRTPYHTGKK
Serca3c 961 P.SGR..GV..QM.....L...A..YLS..HMD.VLGTFMQARSROLPTTSRTPYHTGLA

Serca2b 1020 FVLLIMPLVVVVYSTDTNFSDFMWS
10 Serca3b 1021 GPEVNPGSRGESPVWPSD
Serca3c 1021 SWKKRT

Figure 2

Sequence similarity of Serca2 proteins in mammalian species

	Mouse_2a	1	MENAHTKTVEEVLGHFGVNESTGLSLEQVKKLKERWGSNELPAEEGKTLLLELVIEQFEDL
5	Mouse_2b	1
	Rat_2b	1
	Rat_2a	1
	Dog_2a	1
	Cat_2a	1Y.....
10	Pig_2a	1
	Pig_2b	1
	Human_2b	1
	Human_2c	1
	Human_2a	1
15	Rabbit_2a	1
	Rabbit_2b	1
	Mouse_2a	61	LVRILLLAACISFVLAWFEEGEETITAFVEPFVILLILVANAIVGVWQERNAENAIEALK
	Mouse_2b	61
20	Rat_2b	61
	Rat_2a	61
	Dog_2a	61
	Cat_2a	61
	Pig_2a	61
25	Pig_2b	61
	Human_2b	61
	Human_2c	61
	Human_2a	61
	Rabbit_2a	61
30	Rabbit_2b	61
	Mouse_2a	121	EYEPENMGKVYRQDRKSVQRIKAKDIVPGDIVEIAVGDKVPADIRLTSIKSTTLRVDQSIL
	Mouse_2b	121
	Rat_2b	121
35	Rat_2a	121
	Dog_2a	121
	Cat_2a	121
	Pig_2a	121
	Pig_2b	121
40	Human_2b	121
	Human_2c	121
	Human_2a	121
	Rabbit_2a	121
	Rabbit_2b	121
45	Mouse_2a	181	TGESVSVIKHTDPVPDPRAVNQDKKNMLFSGTNIAAGKAMGVVVATGVNTEIGKIRDEMVA
	Mouse_2b	181
	Rat_2b	181
	Rat_2a	181
50	Dog_2a	181
	Cat_2a	181
	Pig_2a	181
	Pig_2b	181
	Human_2b	181
55	Human_2c	181
	Human_2a	181
	Rabbit_2a	181
	Rabbit_2b	181

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	Mouse_2a	241	ATEQERTPLQQLDEFGEQLSKVISLICIAVWIINIGHFNDFVHGGSWIRGAIYYFKIAV
	Mouse_2b	241
	Rat_2b	241
5	Rat_2a	241
	Dog_2a	241
	Cat_2a	241
	Pig_2a	241
	Pig_2b	241
10	Human_2b	241
	Human_2c	241
	Human_2a	241
	Rabbit_2a	241
	Rabbit_2b	241
15	Mouse_2a	301	ALAVAAIPEGLPAVITTCLALGTRRMAKKNAIVRSLPSVETLGCTSVICSDKTGTLTTNQ
	Mouse_2b	301
	Rat_2b	301
	Rat_2a	301
20	Dog_2a	301
	Cat_2a	301
	Pig_2a	301
	Pig_2b	301
	Human_2b	301
25	Human_2c	301
	Human_2a	301
	Rabbit_2a	301
	Rabbit_2b	301
30	Mouse_2a	361	MSVCRMFIELDKVEGDTCSLNEFSITGSTYAPIGEVQKDDKPKVCHQYDGLVELATICALC
	Mouse_2b	361
	Rat_2b	361T.....
	Rat_2a	361T.....
	Dog_2a	361R...S...T.....H.....
35	Cat_2a	361T.....H.....
	Pig_2a	361T.....H.....
	Pig_2b	361T.....H.....
	Human_2b	361R.....T.....H.....N.....
	Human_2c	361R.....T.....H.....N.....
40	Human_2a	361R.....T.....H.....N.....
	Rabbit_2a	361D.....T.....H.....
	Rabbit_2b	361D.....T.....H.....
45	Mouse_2a	421	NDSALDYNEAKGVYEKVGEATETALTCLVEKMNVDTELKGLSKIERANACNSVIKQLMK
	Mouse_2b	421
	Rat_2b	421
	Rat_2a	421
	Dog_2a	421
	Cat_2a	421K.F.....
50	Pig_2a	421
	Pig_2b	421
	Human_2b	421
	Human_2c	421
	Human_2a	421
55	Rabbit_2a	421
	Rabbit_2b	421

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	Mouse_2a	481	KEFTLEFSRDRKSMSVYCTPNKPSRTSMSKMFVKGAPEGVIDRCTHIRVGSTKVPMTPGV
	Mouse_2b	481
	Rat_2b	481
	Rat_2a	481
5	Dog_2a	481
	Cat_2a	481
	Pig_2a	481
	Pig_2b	481
	Human_2b	481S..
10	Human_2c	481S..
	Human_2a	481S..
	Rabbit_2a	481A..
	Rabbit_2b	481A..
15	Mouse_2a	541	KQKIMSVIREWGSGSDTLRCLALATHDNPLKREEMHLEDSANFTIKYETNLTFVGCVGMLD
	Mouse_2b	541
	Rat_2b	541R.....
	Rat_2a	541R.....
	Dog_2a	541	...V.....R...N.....
20	Cat_2a	541	...V.....R...N.....
	Pig_2a	541MR...N.....
	Pig_2b	541MR...N.....
	Human_2b	541R.....
	Human_2c	541R.....
25	Human_2a	541R.....
	Rabbit_2a	541R.....K.....
	Rabbit_2b	541R.....K.....
30	Mouse_2a	601	PPRIEVASSVKLCRQAGIRVIMITGDNKGTAVAICRRIGIFGQDEDVTSKAFTGREFDEL
	Mouse_2b	601
	Rat_2b	601
	Rat_2a	601
	Dog_2a	601
	Cat_2a	601
35	Pig_2a	601
	Pig_2b	601
	Human_2b	601
	Human_2c	601
	Human_2a	601
40	Rabbit_2a	601E...A.....
	Rabbit_2b	601E...A.....
45	Mouse_2a	661	SPSAQRDACLNARCFARVEPSHKSKIVEFLQSFDEITAMTGDGVNDAPALKKSEIGIAMG
	Mouse_2b	661
	Rat_2b	661
	Rat_2a	661
	Dog_2a	661
	Cat_2a	661
	Pig_2a	661	N.....E.....
50	Pig_2b	661	N.....E.....
	Human_2b	661	N.....A.....
	Human_2c	661	N.....A.....
	Human_2a	661	N.....A.....
	Rabbit_2a	661	N.....A.....
55	Rabbit_2b	661	N.....A.....

	Mouse_2a	721	SGTAVAKTASEMVLADDNFSTIVAAVEEGRAIYNNMKQFIRYLISSNVGEVVCIFLTAAL
	Mouse_2b	721
	Rat_2b	721
	Rat_2a	721
5	Dog_2a	721
	Cat_2a	721
	Pig_2a	721
	Pig_2b	721
	Human_2b	721
10	Human_2c	721
	Human_2a	721
	Rabbit_2a	721
	Rabbit_2b	721
15	Mouse_2a	781	GFPEALIPVQLLWVNLVTDGLPATALGFNPPDLDIMNKPFRNPKEPLISGWLFFRYLAIG
	Mouse_2b	781
	Rat_2b	781
	Rat_2a	781
	Dog_2a	781
20	Cat_2a	781
	Pig_2a	781
	Pig_2b	781
	Human_2b	781
	Human_2c	781
25	Human_2a	781
	Rabbit_2a	781
	Rabbit_2b	781
30	Mouse_2a	841	CYVGAATVGAAAWWFIAADGGPRVSFYQLSHFLQCKEDNPDFDGVDCAI FES PYPMTMAL
	Mouse_2b	841
	Rat_2b	841 E.....
	Rat_2a	841 E.....
	Dog_2a	841 D..... E.....
	Cat_2a	841 D..... E.....
35	Pig_2a	841 T..... E..... V.....
	Pig_2b	841 T..... E..... V.....
	Human_2b	841 E.....
	Human_2c	841 E.....
	Human_2a	841 E.....
40	Rabbit_2a	841 E.....
	Rabbit_2b	841 E.....
45	Mouse_2a	901	SVLVTIEMCNALNSLSENQSLLRMPWENIWLVGSI CLSMLS HFLILYVEPLPLIFQITP
	Mouse_2b	901
	Rat_2b	901
	Rat_2a	901
	Dog_2a	901
	Cat_2a	901
	Pig_2a	901
50	Pig_2b	901
	Human_2b	901
	Human_2c	901
	Human_2a	901
	Rabbit_2a	901
55	Rabbit_2b	901

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Mouse_2a 961 LNL TQWLMVLKISLPVILMDET LKFVARNYLEQPAILE-----
Mouse_2b 961GKECVQPATKSSCSLSACTDGISWPF
Rat_2b 961GKECA.....P.....
Rat_2a 961AILE
5 Dog_2a 961AILE
Cat_2a 961AILE
Pig_2a 961AILE
Pig_2b 961GKEC.....F.....
Human_2b 961 ..V.....GKEC.....F.....
10 Human_2c 961 ..V.....VLSSL
Human_2a 961 ..V.....AILE
Rabbit_2a 961 ..V.....AILE
Rabbit_2b 961 ..V.....GKEC....PQ.-...W...E.V....

15 Mouse_2b 1021 VLLIMPLVVVYSTDTNFSDMEFS
Rat_2b 1020
Pig_2b 1019
Human_2b 1019
Rabbit_2b 1019V...M.....LL..
20

Fig. 3 Targeting construct for Serca2 flox gene modification. Sequence information.

----- Serca2 gene -----

LoxP site 1: Intron 1
 underlined sequence = loxP site and cloning sequence

Exon I (partial)

5 CACGGGGCTGAGCTTGGAGCAGGTCAAGAAGCTCAAGGAGAGATGGGGCTCCAACGgtaggtgcggggcg
 ccggggcgtgcagcggcgcgggcgggcccgagcgccaaggaagatggctgacccgggtccacccctcgtggg
 gcttggctcggcgcgggcgcccgacggctgcgagagggccggcggtccacgcggggtctgggccaatcgccg
 10 accttaggggtctcgaatcaagcttatcgataccgtcgatcggaacctcgagggggggcccggtacccggg
 gatcaattcgagctcgcccggggatcgatccggaaccttaataATAACTTCGTATAATGTATGCTATACG
 AAGTTATtaggtccctcgacctgcagcccaagctccGGGGAatctcgagccgggtgaccttcccgccggcg
 ctgagcgagtcggattgggggggggggagagggagtggaaggaggaggaggttcttcgggctgggctg
 agtcccccgcgatttatgaggcgctcgatggttggtagaaaccttcggacctttcttctgctccccaaa
 15 gttgcacatctggcagaagtgatgaccagctgaaatgactgcatggctctggaggccggagagggctta
 cgggcagttccgaggccactgattaccagggctgaataattttctcggggtatcaaagtggagacagatt
 gttgtacgttcatacacctatatccgccattcagacaacgatgggtggtagaatttagcagtttttaataaa
 agcgctaatacaatatcttcattttttctttc

----- Serca2 gene -----

LoxP site 2: Intron 3 5' of genomic XcmI site
 underlined sequence = loxP site, cloning sites and partial HSV-TK

20 ccaatttttattcttagaacaattgtattcttatactgtgataggaagtgaataatcatacagtaacttgtc
 ttagggtttcacaaaaactgataactgtatgggttcaattatgtattcacacgtttaagtctgacccagggG
 GATCCggaaccttaataATAACTTCGTATAATGTATGCTATACGAAGTTATtaggtccctcgacctgcag
 25 cccaagctgatccctctagtcgagcccgagctgggttctttccgcctcagaagccatagagcccccagaata
 ccagcatgcctgctattgtcttcccaatccctcccttgcctgcttgcctgccccacccccacccccagaata
 gaatgacacctactcagacaatgcgatgcaatttctctcattttatttaggaaaggacagtgaggagtggcac
 cttccaggggtcaaggaaggcacgggggaggggcaaacacagatggctggcaactagaaggcacagtcga
 30 ggctgatcagcgagctctagctagagaattgatccctcagaagaactcgtcaagaaggcgatagaaggc
 gatgcgctgcgaatcgggagcggcgat*ccgtaaaagcacgaggaagcgg*cgcccatctcgccccaagc
 tctttcagcaatatcacgggtagccaacgctatgt*ctgataagcgggtccgcccacacccaa*ccggcca
 caagtc*atgaaatcca*aaaaagcgggcccatttttccacc*atgatttt*cgqcaagcaaggccctt*c
 cattgggtcaccgac*aga*catt*tcctgt*c*ggcattgcgc*ccct

----- HSV-TK Neo antibiotics cassette -----

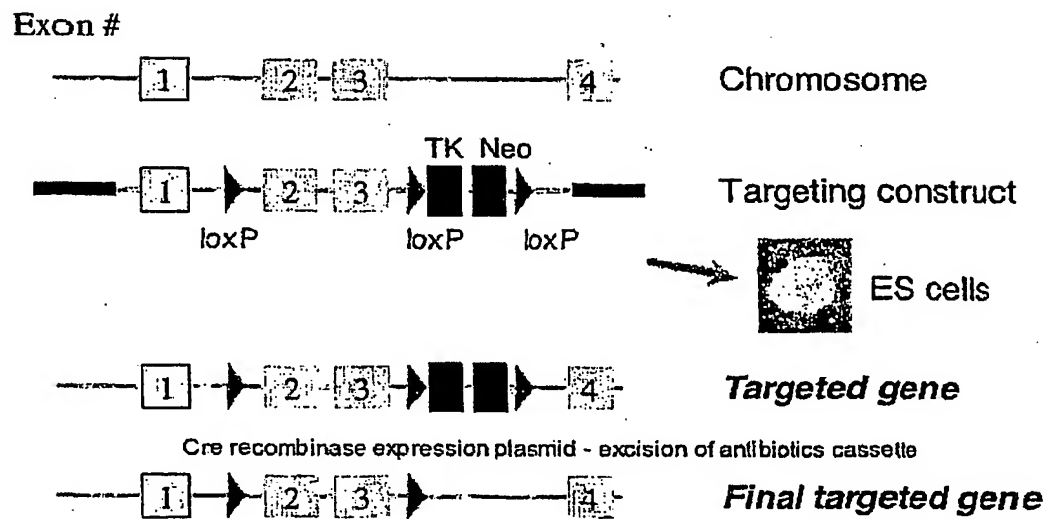
LoxP site 3: Intron 3 5' of genomic XcmI site
 underlined sequence = loxP site, cloning sites and partial Neo gene

35 gttttcat*accaccgcgggtcccgggc*gatat*ttcaccttctc*ag*cggtgttgtgtggtgtaaatg
 ttcgcgatttgttcgaaagcccc*agcaccgcagtaagtcacatcggtcgggtacgtagacgatatcgctc
 gcgcgaacccagggccaccagcaagttgcgtggtggtggttttcccatcc*gtggggac*gtctatataa
 40 acc*gcagtagcgtgggcattttctgctcgggcccgaacttccgtggcttcttgcctgcccggcgagggcgcaa
 cggcgtacgtcggttgcctatggccgcgagaaacgcgcagcctgggtcgaacgcagacgcgtgttgatggccgg
 ggtacgaagccatacgcgcttctacaaggcgcgtggccgaagaggtgcgggagtttcacgccaccaagatct
 gcggcacgctgttgacgctgttaagcgggtcgctgcagggctcgctcggtgttcgaggccacacgcgtcacc
 ttaatatgcgaagtggacctcggaaccgcgcgccccgactgcatctgcgtgttcgaattcgccaatgacaa
 45 gacgctggggcggggtttgctcgacattgggtggaaacattccaggcctgggtggagaggcctttttgcttcc
 tcttgcaaaaccacactgctcgacattgggtggaaacattccaggcctgggtggagaggcctttttgcttcc
 tcttgcaaaaccacactgctcgacattgggtggaaacattccaggcctgggtggagaggcctttttgcttcc
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 acattagcttagagg

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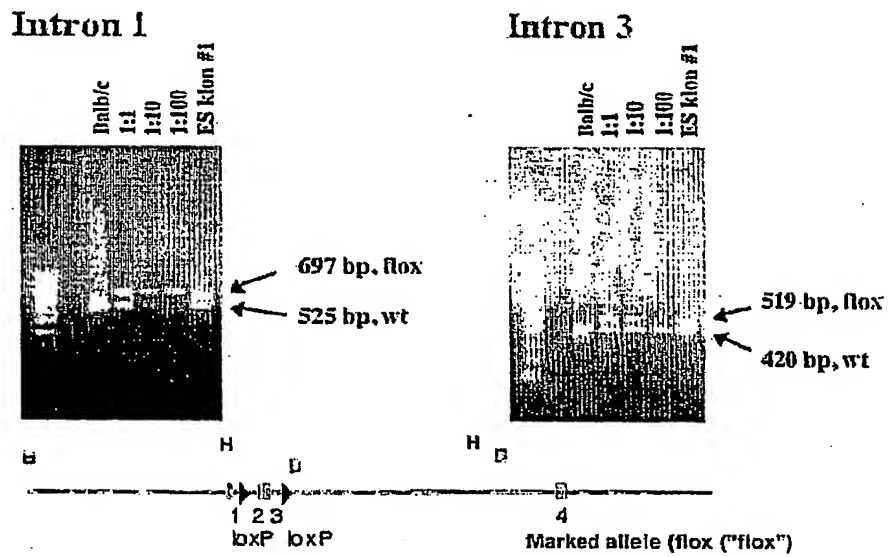
Fig. 4A Schematic representation of genetic manipulation.

Serca2 (*atp2a2*) gene modification



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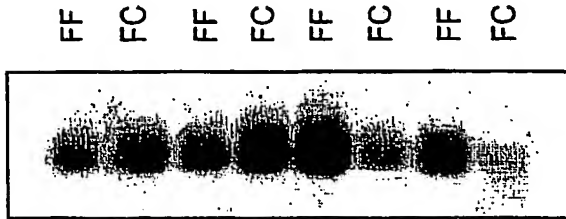
Fig 4 B: Verification of Serca locus targeting events offspring from chimeric mice.



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Fig. 6 Cardiac ANP mRNA expression.

5

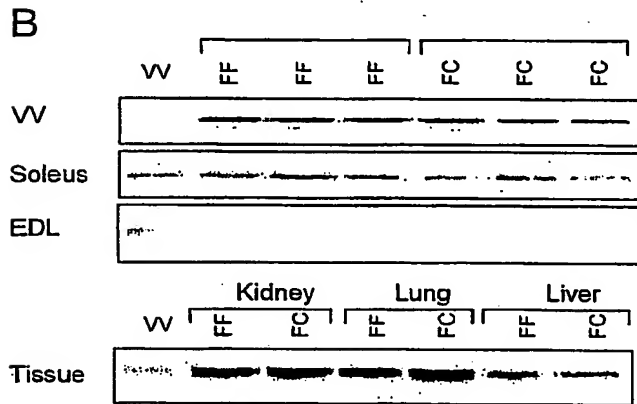
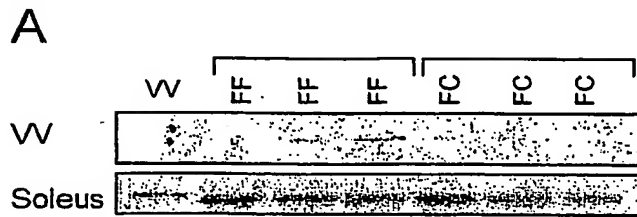


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Fig. 7 Serca2 protein expression.

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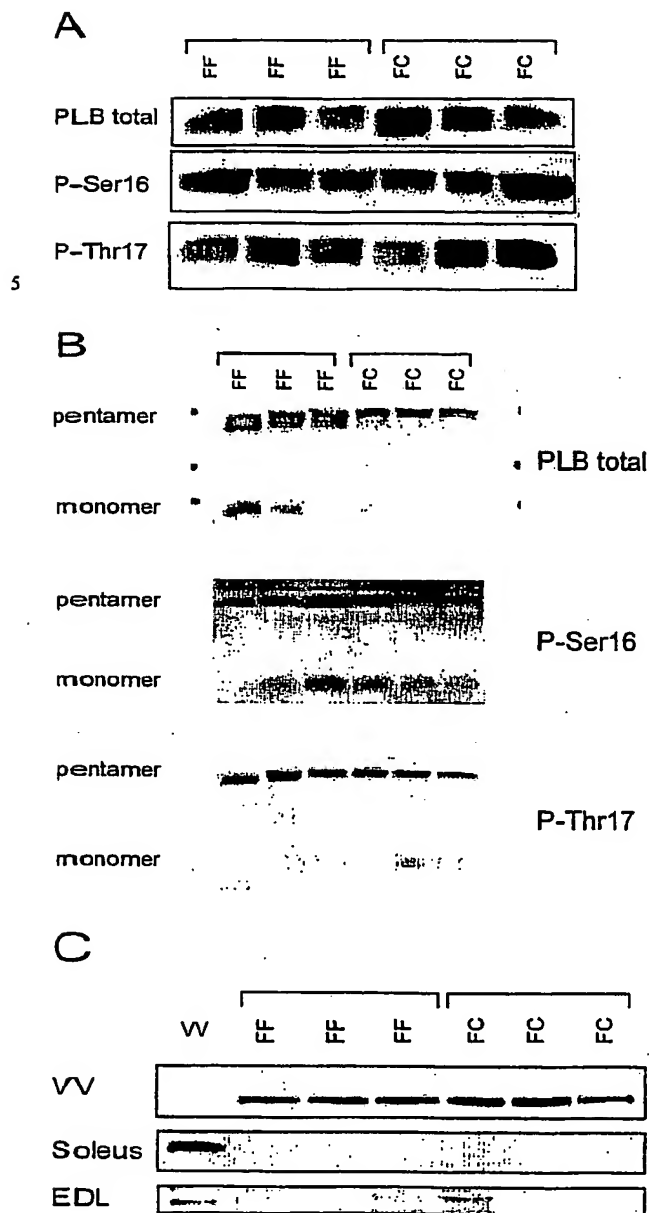


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Fig. 8 Compensatory mechanisms in *Serca^{flox}* MLC-2 ν -Cre mice.

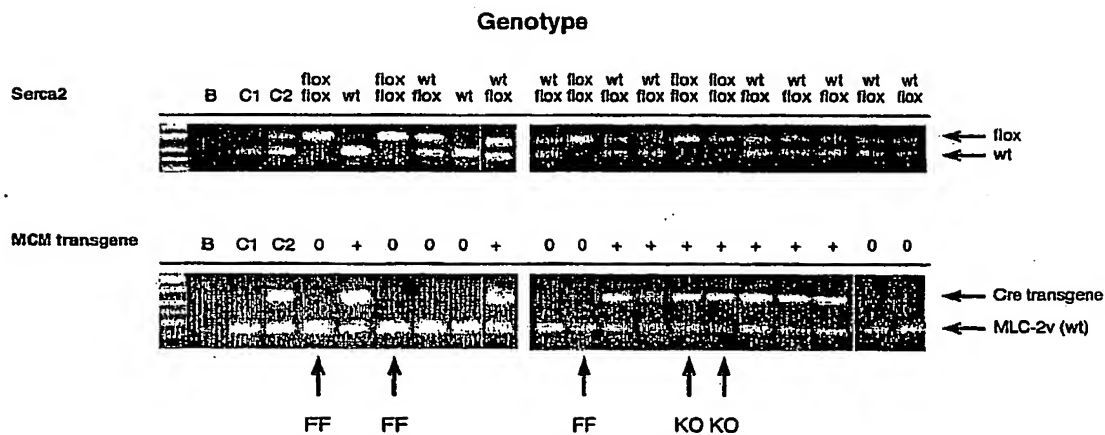


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DECT A1111 ADIE C0DV

Figure 9 Genotypes PCR

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10 Generation of animals with Serca2^{flox} and MCM transgene alleles.

Genotypes FF. Serca2^{flx/flx}, KO, Serca2^{flx/flx} MCM

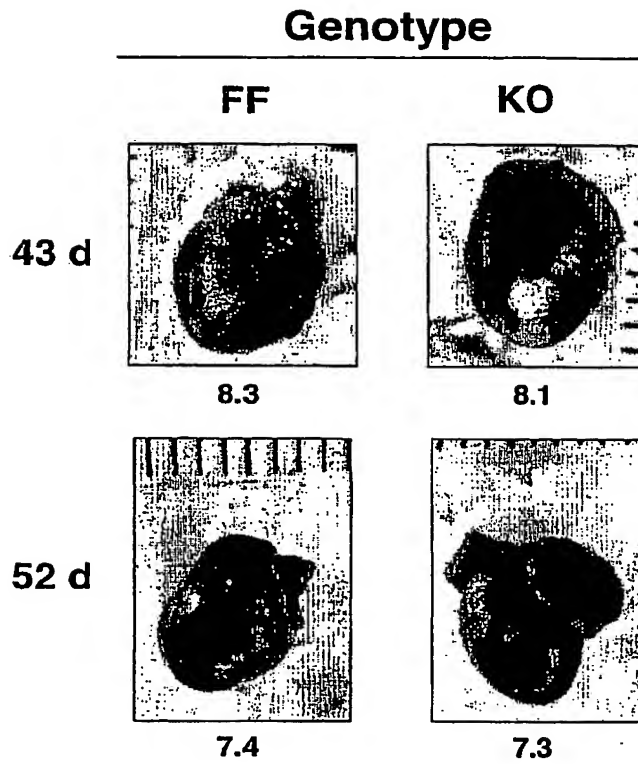
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Figure 10

Heart morphology

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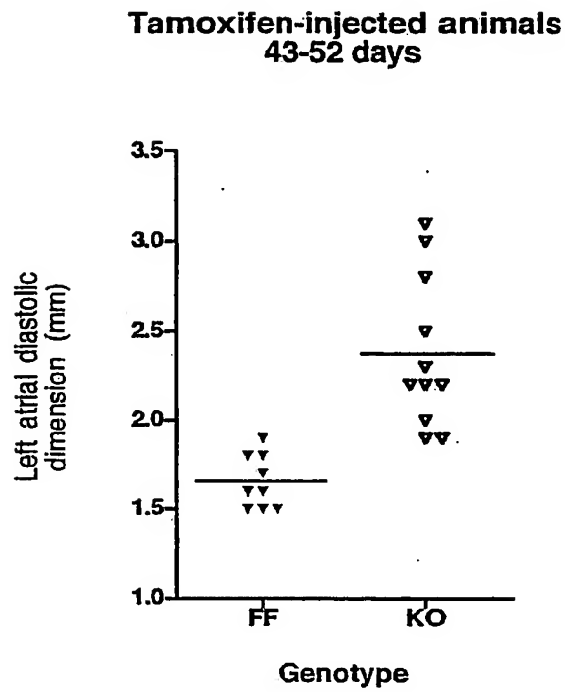
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Figure 11 Pilot series left atrial diastolic diameter.

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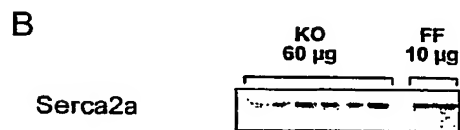
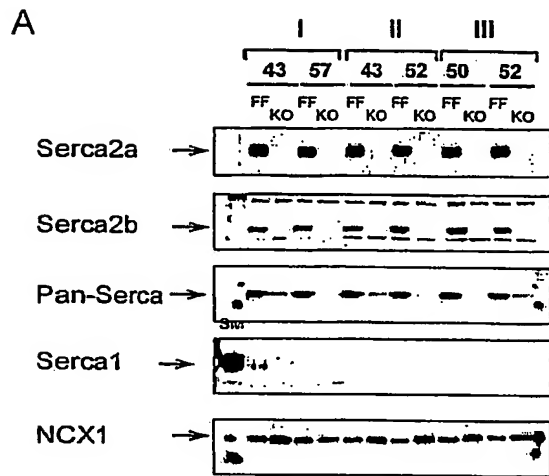
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Figure 12 Serca protein content in tamoxifen-induced FF and KO mice

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